Journal of Medical Virology 57:75–79 (1999)

High Prevalence of GB Virus C/Hepatitis G Virus RNA in Patients Infected With Human Immunodeficiency Virus

D. Rey,[1]* S. Fraize,[1] J. Vidinic,[2] P. Meyer,[3] S. Fritsch,[2] N. Labouret,[2] C. Schmitt,[2] J.M. Lang,[1] and F. Stoll-Keller[2]

[1]*CISIH, Clinique Médicale A, Hôpitaux Universitaires, Strasbourg, France*
[2]*Institut de Virologie, Faculté de Médecine, Strasbourg, France*
[3]*Département d'Informatique Médicale, Hôpitaux Universitaires, Strasbourg, France*

Prevalence of GBV-C/HGV was determined in a cohort of HIV-infected patients, via a reverse transcription–polymerase chain reaction detection of RNA in serum, amplifying the NS5 region of GBV-C/HGV genome. GBV-C/HGV RNA was detected in 143 (37.7%) of 379 patients, with similar results in the different HIV risk groups: 25/56 (44.6%) in intravenous drug users, 66/161 (41%) in homo- and bisexual men, 35/108 (32.4%) in heterosexual patients, 6/20 (30%) in transfusion recipients ($P = 0.41$). There was no difference according to the presence or absence of hepatitis C virus infection. In univariate analysis, GBV-C/HGV genome prevalence was lower in patients over 50 years old (18.2%), compared to other age groups (20–29 years: 34.2%; 30–39 years: 44.3%; 40–49 years: 36.7%, $P = 0.03$), as well as in patients with normal CD4 cell count (29.2% vs. 45.4% between 200–500/mm^3, and 35.3% below 200 CD4/mm^3, $P = 0.012$) and individuals with a chronic hepatitis B. However, in the multivariate analysis, the only prognostic factor of GBV-C/HGV RNA positivity was the presence of a chronic hepatitis B, compared to the absence of any HBV marker, or a previous exposition to HBV (presence of anti-HBc and/or anti-HBs, absence of HBsAg), or the presence of anti-HBs alone. ***J. Med. Virol. 57:75–79, 1999.***

KEY WORDS: GBV-C/HGV; HIV infection; hepatitis B virus; polymerase chain reaction; parenteral exposure; sexual exposure

INTRODUCTION

GB virus C (GBV-C)/hepatitis G virus (HGV), potential agents of viral hepatitis, were recently identified by molecular biology methods [Simons et al., 1995; Linnen et al., 1996; Muerhoff et al., 1997a]; they belong to the *Flaviviridae* family [Leary et al., 1996] and their genome consists in a positive-strand RNA of about 10 kb that encodes the structural proteins at the 5′ end and the nonstructural proteins at the 3′ end of the genome [Simons et al., 1995; Leary et al., 1996; Linnen et al., 1996]. The nucleotide sequences are homologous (86%), indicating that they are closely related variants of the same virus [Berg et al., 1996; Zuckerman, 1996; Yashina et al., 1997]. Phylogenetic analysis showed that the GBV-C genome was constrained; as a result, there is a high degree of nucleotide and amino acid sequence conservation between isolates from different geographical areas [Muerhoff et al., 1997b]. The clinical effect of GBV-C/HGV, which is still debated, seems to be very low [Alter MJ et al., 1997; Feucht et al., 1997a; Martinot et al., 1997; Sampietro et al., 1997; Skidmore et al., 1997; Thomas et al., 1997; Vargas et al., 1997; Yashina et al., 1997]. Replication in hepatocytes is controversial [Laskus et al., 1997; Saito et al., 1997], since it is also found in peripheral blood mononuclear cells [Saito et al., 1997; Radkowski et al., 1998].

However, many epidemiological studies have been undertaken, usually by serum RNA detection, showing a high prevalence of this virus in humans: 0.8–13% in blood donors [Dawson et al., 1996; Linnen et al., 1996; Stark et al., 1996; Feucht et al., 1997a; Wang and Jin, 1997], much more higher rates in patients with parenteral exposures (intravenous drug users [Dawson et al., 1996; Linnen et al., 1996; Schreier et al., 1996; Stark et al., 1996; Feucht et al., 1997b; Fong et al., 1997; Thomas et al., 1997], transfusion recipients [Linnen et al., 1996; Alter HJ et al., 1997; Feucht et al., 1997b; Sampietro et al., 1997; Skidmore et al., 1997], hemophiliacs

*Correspondence to: D. Rey, CISIH, Clinique Médicale A, Hôpitaux Universitaires, 1, place de l'Hôpital, 67091 Strasbourg Cedex, France. E-mail: david.rey@chru-strasbourg.fr

Accepted 25 June 1998

[Jarvis et al., 1996; Linnen et al., 1996; Nübling and Löwer, 1996; Alter HJ et al., 1997; Feucht et al., 1997b; Kinoshita et al., 1997], and patients on chronic hemodialysis [Masuko et al., 1996; Tsuda et al., 1996; Feucht et al., 1997b; Lampe et al., 1997; Ross et al., 1998]). Sexual transmission also occurred [Stark et al., 1996; Rubio et al., 1997], as well as vertical transmission [Feucht et al., 1996; Fischler et al., 1997; Hino et al., 1998]. GBV-C/HGV may also be frequently found in patients with acute non-A–E hepatitis [Alter MJ et al., 1997; Kao et al., 1997; Wang and Jin, 1997], chronic hepatitis B [Linnen et al., 1996; Alter MJ et al., 1997; Kao et al., 1997; Wang and Jin, 1997] or C [Berg et al., 1996; Bhardwaj et al., 1996; Dawson et al., 1996; Linnen et al., 1996; Tanaka et al., 1996; Alter HJ et al., 1997; Alter MJ et al., 1997; Feucht et al., 1997b; Kao et al., 1997; Wang and Jin, 1997; Yashina et al., 1997; Manolakopoulos et al., 1998], or with hepatocellular carcinoma [Linnen et al., 1996; Kao et al., 1997; Wang and Jin, 1997]; nevertheless, it does not seem to affect the progression of the associated hepatitis.

On the other hand, few studies were conducted in immunocompromised individuals, especially in HIV-infected patients. Moreover the role of the immune impairment on GBV-C/HGV RNA detection is unknown. The aim of the present study was to determine the prevalence of GBV-C/HGV, by genome detection, in a cohort of HIV-infected patients, and to examine the factors that could influence this prevalence.

MATERIALS AND METHODS

Patients

A total of 397 consecutive HIV-infected patients were included in the study, from 1 December 1996 to 31 May 1997. They were all followed in an outpatient clinic (Centre d'Informations et de Soins de l'Immunodéficience Humaine, Hôpitaux Universitaires, Strasbourg, France). HIV-1 infection was confirmed by a Western blot assay (New LAV blot I, Sanofi Diagnostics Pasteur, Marmes la Coquette, Francé). The following demographic data were recorded: age, sex, HIV risk group, and Centers for Disease Control (CDC) 1993 classification. In addition, CD4 lymphocyte cell count was measured by flow cytometry; the last result before hepatitis G virus (HGV) testing was noted. The mean age of the cohort was 36.8 years.

Hepatitis Serology

Sera were tested for hepatitis C virus (HCV) antibodies by third-generation commercially available ELISAs, and also for hepatitis B virus (HBV) markers (HBsAg, anti-HBs, anti-HBc, HBeAg, and anti-HBe). Patients were considered to have chronic hepatitis B if they had an HBsAg (with or without the HBeAg); they were considered to have been exposed to HBV if they had antibodies to hepatitis B core antigen (anti-HBc), anti-HBs, but were HBsAg-negative; patients with anti-HBs alone were also separated as a group (probable hepatitis B immunization).

GBV-C/HGV RNA Detection

Serum was obtained by centrifugation of the blood sample at 2,500 rounds/min for 10 min, within 6 hr of venous puncture, and stored at −80°C. RNA was extracted from 100 μl of thawed serum with 400 μl of lysis buffer (HCV Amplicor Roche, Roche Diagnostic, Meylan, France) at 60°C for 10 min. Five hundred μl of isopropanol were added, followed by a centrifugation at 14,000 rounds/min for 15 min, and a washing with 1 ml of ethanol 70%. The resulting pellet was suspended in 25 μl of DEPC water. RNA was reverse-transcribed and amplified by use of primers from the NS5 region of GBV-C/HGV. Seven μl of RNA were first reverse-transcribed with 40 pmoles of antisense primer 211R (5′-CGAATGAGTCAGAGGACGGGGTAT-3′) in 9 μl of RT master mix (4 μl of $MgCl_2$ 25 mM, 2 μl of dNTP 200 M, 2 μl of 10 × Perkin Elmer buffer, 1 μl of Perkin Elmer Moloney murine leukemia virus reverse transcriptase) at 42°C for 15 min. Fifteen μl of the resulting cDNA were amplified with 85 μl of PCR master mix (10 μl of Perkin Elmer buffer, 5 μl of $MgCl_2$ 25 mM, 5 μl of sense primer 77F [5′-CTCTTTGTGGTAGTAGCCGAGAGAT-3′], 2 μl of dNTP 200 M, 2 μl of Perkin Elmer Taq DNA polymerase).

Amplification was run as follows: initial denaturing of 94°C for 5 min, followed by 35 cycles of 94°C (1 min), 60°C (1 min), and 72°C (1 min), and a final extension of 72°C for 10 min (HYBAID Omnigen thermocycler, Omnigen, UK). Twenty-five μl of the PCR product were analyzed by electrophoresis in a 2.5% agarose gel, with ethidium bromide staining, and visualized under ultraviolet illumination (showing a 155 base pair). The specificity of the PCR product was confirmed by hybridization in liquid medium (152F probe, DEIA Sorin Biomedica kit, Paris, France). Each sample was tested in duplicate; a PCR was considered positive or negative should both reactions of the same sample give the same result, and indeterminate should one reaction be positive and the other negative.

Statistical Analysis

The chi-square test was used to assess the association between GBV-C/HGV and the demographic variables, the CD4 lymphocyte count, and HBV and HCV status. A logistic regression analysis was also done to evaluate the prognostic value of the different variables and to adjust for potential confounding factors.

RESULTS

Of 397 patients, 143 positive and 236 negative results were obtained, as well as 18 indeterminate reactions, which were excluded from statistical analysis. Thus, the overall GBV-C/HGV prevalence was 37.7% (143/379), similar in male and female patients (37.9% vs. 37.1%, $P = 0.88$).

GBV-C/HGV genome detection was slightly more frequent in patients with an AIDS diagnosis: 48/136 (35.3%) in group A, 76/196 (38.8%) in group B, and 19/47 (40.4%) in group C, but the difference was not

TABLE I. GBV-C/HGV Prevalence According to HIV Risk Group

HIV risk group	GBV-C/HGV–positive number (%)	GBV-C/HGV–negative number (%)
Intravenous drug users	25 (44.6)	31 (55.4)
Homosexual men	66 (41)	95 (59)
Heterosexual patients	35 (32.4)	73 (67.6)
Transfusion recipients	6 (30)	14 (70)
Indeterminate	11 (33.3)	22 (66.6)

TABLE II. GBV-C/HGV Prevalence in Different Age Groups

Age, years	GBV-C/HGV–positive number (%)	GBV-C/HGV–negative number (%)
20–29	27 (34.2)	52 (65.8)
30–39	77 (44.3)	97 (55.7)
40–49	33 (36.7)	57 (63.3)
>50	6 (18.2)	33 (81.8)

TABLE III. GBV-C/HGV Prevalence According to HBV Status

HBV status	GBV-C/HGV–positive number (%)	GBV-C/HGV–negative number (%)
Chronic hepatitis B infection	19 (57.6)	14 (42.4)
No HBV marker	49 (40.2)	73 (59.8)
Previous exposition to HBV[a]	63 (35.8)	113 (64.2)
Anti-HBs alone	10 (22.7)	34 (77.3)

[a]Patients with anti-HBc, anti-HBs, without HBsAg.

significant ($P = 0.75$) according to this clinical classification. However, GBV-C/HGV RNA prevalence was lower in patients with a normal CD4 cell count result (>500/mm^3), 40/137 (29.2%), compared to patients with a moderate immune deficit (CD4 between 200 and 500/mm^3), 79/174 (45.4%), and individuals with a severe immune deficit ($CD_4 < 200/mm^3$), 24/68 (35.3%). This difference is significant ($P = 0.012$). Thus GBV-C/HGV genome prevalence was not influenced by the clinical status, but by the immune (CD4 cell count) status.

Table I shows the results according to the HIV risk group; it varies from 30% to 44.6%, but the differences were not significant ($P = 0.41$). Thus, GBV-C/HGV prevalence was high, but not different, between sexual (homo- and heterosexual) and parenteral exposures. GBV-C/HGV genome prevalence was lower in older patients (over 50 years) compared to the other age groups (Table II). This difference reached a statistical significance ($P = 0.03$). Coinfection with HCV did not influence GBV-C/HGV prevalence: 34/78 (43.6%) in patients coinfected with HIV and HCV, vs. 107/298 (35.9%) in patients infected with HIV without HCV infection ($P = 0.21$). On the other hand, it was increased by HBV (Table III). Indeed, GBV-C/HGV prevalence was higher in patients with a chronic hepatitis B, and the lowest rate was observed in patients with anti-HBs alone ($P = 0.016$).

In the multivariate analysis, the presence of a chronic hepatitis B infection was the only significant factor increasing GBV-C/HGV prevalence (OR = 2.56; 95% CI, 1.19–5.47).

DISCUSSION

In this epidemiological study, a high prevalence (37.7%) of GBV-C/HGV RNA was found in an HIV-infected cohort of 397 patients, using an RT-PCR detection of RNA amplifying the NS5 region. Several studies of GBV-C/HGV prevalence in HIV-infected patients have been published; a high frequency of GBV-C/HGV genome detection was also found: 10/55 (18.2%) in individuals with HIV-infection, but HIV risk groups weren't specified [Feucht et al., 1997b], 9/100 (9%) in intravenous drug users and homosexual men [Nübling and Löwer, 1996], 8/33 (24%) in HIV-infected hemophiliacs [Kinoshita et al., 1997], 3/17 (17.6%) in HIV-infected pregnant women [Feucht et al., 1996], 2/20 (10%) in another series of HIV-seropositive hemophiliacs [Jarvis et al., 1996], and 11/41 (26.8%) in Japanese hemophilia patients with HIV coinfection [Toyoda et al., 1998].

In our study, the results of HIV-infected patients were not compared to a non-HIV-infected cohort. However, it seemed that HIV by itself did not increase GBV-C/HGV prevalence. For instance, Stark et al. [1996] did not find any association of GBV-C/HGV prevalence with HIV antibodies in intravenous drug users and homosexual men; 24% of hemophiliacs with HIV antibodies, vs. 23% without HIV antibodies, were GBV-C/HGV–positive for Kinoshita et al. [1997]; Jarvis et al. [1996] found GBV-C/HGV RNA in 10% of HIV-infected hemophiliacs vs. 11/75 (14.7%) of HIV-negative hemophiliacs; GBV-C/HGV RNA status was not associated with HIV in 246 intravenous drug users (OR, 0.9 ; 95% CI; 0.4–2.0) [Thomas et al., 1997]; GBV-C/HGV RNA was detected in 11% and 8%, respectively, of HIV-positive and HIV-negative patients (81 hemophiliacs) [Goedert et al., 1997].

GBV-C/HGV prevalence is linked, as shown in several studies, to sexual and parenteral exposures: detection of GBV-C/HGV RNA in 11% and 13.4% of homo- and bisexual men [Stark et al., 1996; Rubio et al., 1997], 13.9% of female prostitutes [Rubio et al., 1997], 5/23 (21.7%) of heterosexual partners of GBV-C/HGV–infected individuals [Rubio et al., 1997]; GBV-C/HGV RNA prevalence of 3.1% to 55% in dialysis patients [Masuko et al., 1996; Tsuda et al., 1996; Feucht et al., 1997b; Lampe et al., 1997; Ross et al., 1998], 14% to 35.2% in hemophiliacs [Linnen et al., 1996; Jarvis et al., 1996; Feucht et al., 1997b; Kinoshita et al., 1997], 10% to 48% in transfusion recipients [Linnen et al., 1996; Alter HJ et al., 1997; Feucht et al., 1997b; Sampietro et al., 1997; Skidmore et al., 1997], including thalassemia major patients [Sampietro et al., 1997] and bone marrow transplant recipients and patients with acute leukemia [Skidmore et al., 1997], 9.7% to 49% in intravenous drug users [Dawson et al., 1996; Linnen et al., 1996; Schreier et al., 1996; Stark et al., 1996;

Feucht et al., 1997b; Fong et al., 1997; Thomas et al., 1997].

Interestingly, a lower prevalence of GBV-C/HGV RNA was found in older patients (>50 years) by the chi-square test; however, this difference disappeared in the multivariate analysis, suggesting the role of other factors in the results of the univariate analysis. Nevertheless, the study of Thomas et al. [1997] showed a higher rate of GBV-C/HGV RNA detection in younger intravenous drug users (from around 50% in users 20–24 years old, to 5% in users over 39 years old, $P < 0.001$ for trend), as well as among intravenous drug users with shorter durations of drug use. This implies that GBV-C/HGV is a less persistent virus than HCV. Tanaka et al. [1996] also found that patients with GBV-C/HGV infection were significantly younger than those without GBV-C/HGV infection (46.6 years vs. 51.7 years, $P = 0.043$). For Manolakopoulos et al. [1998], patients coinfected with HCV and GB virus C were younger (38.4 years) than patients with HCV alone (42.4 years, $P = 0.04$). On the other hand, Dawson et al. [1996] found an increasing prevalence of GBV-C/HGV RNA with age among west African residents.

To our knowledge, this is the first study to evaluate in HIV-infected individuals the prevalence of GBV-C/HGV genome according to the immune status, reflected by the result of the CD4 cell count. Like the results among the age groups, a significant difference was found by the chi-square test (lower prevalence in patients with a normal CD4 cell count), disappearing in the multivariate analysis. Again, this implies the intervention of confounding factors. However, it might be interesting to complete this study by a follow-up of GBV-C/HGV genome and GBV-C/HGV antibody screening among GBV-C/HGV genome-negative patients.

No correlation was found between GBV-C/HGV prevalence and hepatitis C infection, which is probably due to the high-risk exposure to GBV-C/HGV of all the patients, either parenterally, or sexually, independent of HCV. The rate of GBV-C/HGV coinfection with HCV varies from 8.2% to 44% [Berg et al., 1996; Dawson et al., 1996; Linnen et al., 1996; Tanaka et al., 1996; Alter HJ et al., 1997; Alter MJ et al., 1997; Bhardwaj et al., 1997; Feucht et al., 1997b; Wang and Jin, 1997; Yashina et al., 1997; Manolakopoulos et al., 1998].

The most important prognostic factor of GBV-C/HGV RNA positivity (and the only one in the multivariate analysis), was the presence of a chronic hepatitis B (at least HBsAg carrier, compared to patients with anti-HBc but without HBsAg, individuals without any contact with HBV, and patients with anti-HBs alone [presumably after vaccination]). For GBV-C/HGV-HBV coinfection, discordant results were found in the literature; GBV-C/HGV RNA prevalence in hepatitis B chronic infection may be low: 0/48 for Wang and Jin [1997] and 7/220 (3.2%) for Kao et al. [1997], or high in other studies: 7/72 (9.7%) for Linnen et al. [1996], 32/100 (32%) for Alter MJ et al. [1997]. These last results strongly suggest that HBV and GBV-C/HGV share common routes of transmission. The low coinfection prevalence in the study of Kao et al. [1997] was explained by a specific epidemiological situation in Taiwan: HBV infection is usually contracted during perinatal periods, or early childhood, and GBV-C/HGV superinfection may occur later. They also found that GBV-C/HGV prevalence increased with the severity of liver disease due to HBV (1% in asymptomatic HBsAg carriers and 10% in hepatocellular carcinoma).

ACKNOWLEDGMENTS

We thank Ms. D. Bera, Mr. L. Soley, and Dr. K. Langley for advice on the English version of this article.

REFERENCES

Alter HJ, Nakatsuji Y, Melpolder J, Wages J, Wesley R, Shih JWK, Kim JP. 1997. The incidence of transfusion-associated hepatitis G virus infection and its relation to liver disease. N Engl J Med 336:747–754.

Alter MJ, Gallagher M, Morris TT, Moyer LA, Meeks EL, Krawczynski K, Kim JP, Margolis HS. 1997. Acute non-A–E hepatitis in the United States and the role of hepatitis G virus infection. N Engl J Med 336:741–746.

Berg T, Dirla U, Naumann U, Heuft HG, Küther S, Lobeck H, Schreier E, Hopf U. 1996. Responsiveness to interferon alpha treatment in patients with chronic hepatitis C coinfected with hepatitis G virus. J Hepatol 25:763–768.

Bhardwaj B, Qian K, Detmer J, Mizokami M, Kolberg JA, Urdea MS, Schlauder G, Linnen JM, Kim JP, Davis GL, Lau JYN. 1997. Detection of GB virus C/hepatitis G virus RNA in serum by reverse transcription polymerase chain reaction. J Med Virol 52:92–96.

Dawson GJ, Schlauder GG, Pilot-Matias TJ, Thiele D, Leary TP, Murphy P, Rosenblatt JE, Simons JN, Martinson FEA, Gutierrez RA, Lentino JR, Pachucki C, Muerhoff AS, Widell A, Tegtmeier G, Desai S, Mushahwar IK. 1996. Prevalence studies of GB virus C infection using reverse transcriptase–polymerase chain reaction. J Med Virol 50:97–103.

Feucht HH, Zöllner B, Polywka S, Laufs R. 1996. Vertical transmission of hepatitis G. Lancet 347:615–616.

Feucht HH, Zöllner B, Polywka S, Knodler B, Schroter M, Nolte H, Laufs R. 1997a. Distribution of hepatitis G viremia and antibody response to recombinant proteins with special regard to risk factors in 709 patients. Hepatology 26:491–494.

Feucht HH, Zöllner B, Polywka S, Knödler B, Schröter M, Nolte H, Laufs R (1997b): Prevalence of hepatitis G viremia among healthy subjects, individuals with liver disease, and persons at risk for parenteral transmission. J Clin Microbiol 35:767–768.

Fischler B, Lara C, Chen M, Sönnerborg A, Nemeth A, Sällberg M. 1997. Genetic evidence for mother-to-infant transmission of hepatitis G virus. J Inf Dis 176:281–285.

Fong TL, Lee SR, Kim JP, Yun AJ, Zheng J, Peterson J, Kochesky R, Botla R, Geller SA, Redeker AG. 1997. Prevalence of hepatitis G virus among intravenous drug abusers in Los Angeles. Clin Inf Dis 25:165–166.

Goedert JJ, Steinmeyer LA, Nakatsuji Y. 1997. Hepatitis G infection. N Engl J Med 337:277.

Hino K, Moriya T, Ohno N, Takahashi K, Hoshino H, Ishiyama N, Katayama K, Yoshizawa H, Mishiro S. 1998. Mother-to-infant transmission occurs more frequently with GB virus C than hepatitis C virus. Arch Virol 143:65–72.

Jarvis LM, Davidson F, Hanley JP, Yap PL, Ludlam CA, Simmonds P. 1996. Infection with hepatitis G virus among recipients of plasma products. Lancet 348:1352–1355.

Kao JH, Chen PJ, Lai MY, Chen W, Liu DP, Wang JT, Shen MC, Chen DS. 1997. GB virus-C/hepatitis G virus infection in an area endemic for viral hepatitis, chronic liver disease, and liver cancer. Gastroenterology 112:1265–1270.

Kinoshita T, Miyake K, Nakao H, Tanaka T, Tsuda F, Okamoto H, Miyakawa Y, Mayumi M. 1997. Molecular investigation of GB virus C infection in hemophiliacs in Japan. J Inf Dis 175:454–457.

Lampe E, Saback FL, Yoshida CFT, Niel C. 1997. Infection with GB

virus C/hepatitis G virus in Brazilian hemodialysis and hepatitis patients and asymptomatic individuals. J Med Virol 52:61–67.

Laskus T, Radkowski M, Wang LF, Vargas H, Rakela J. 1997. Lack of evidence for hepatitis G virus replication in the livers of patients coinfected with hepatitis C and G viruses. J Virol 71:7804–7806.

Leary TP, Muerhoff AS, Simons JN, Pilot-Matias TJ, Erker JC, Chalmers ML, Schlauder GG, Dawson GJ, Desai SM, Mushahwar IK. 1996. Sequence and genomic organization of GBV-C: A novel member of the *Flaviviridae* associated with human non-A–E hepatitis. J Med Virol 48:60–67.

Linnen J, Wages J Jr, Zhang-Keck ZY, Fry KE, Krawczynski KZ, Alter H, Koonin E, Gallagher M, Alter M, Hadziyannis S, Karayiannis P, Fung K, Nakatsuji Y, Shih JWK, Young L, Piatak M Jr, Hoover C, Fernandez J, Chen S, Zou JC, Morris T, Hyams KC, Ismay S, Lifson JD, Hess G, Foung SKH, Thomas H, Bradley D, Margolis H, Kim JP. 1996. Molecular cloning and disease association of hepatitis G virus: A transfusion-transmissible agent. Science 271:505–508.

Manolakopoulos S, Morris A, Davies S, Brown D, Hajat S, Dusheiko G. 1998. Influence of GB virus C viraemia on the clinical, virological and histological features of early hepatitis C-related hepatic disease. J Hepatol 28:173–178.

Martinot M, Marcellin P, Boyer N, Detmer J, Pouteau M, Castelnau C, Degott C, Aupérin A, Collins M, Kolberg J, Wilber J, Benhamou JP, Erlinger S. 1997. Inflence of hepatitis G virus infection on the severity of liver disease and response to interferon in patients with chronic hepatitis C. Ann Int Med 126:874–881.

Masuko K, Mitsui T, Iwano K, Yamazaki C, Okuda K, Meguro T, Murayama N, Inoue T, Tsuda F, Okamoto H, Miyakawa Y, Mayumi M. 1996. Infection with hepatitis GB virus C in patients on maintenance hemodialysis. N Engl J Med 334:1485–1490.

Muerhoff AS, Leary TP, Desai SM, Mushahwar IK. 1997a. Amplification and substraction methods and their application to the discovery of novel human viruses. J Med Virol 53:96–103.

Muerhoff AS, Smith DB, Leary TP, Erker JC, Desai SM, Mushahwar IK. 1997b. Identification of GB virus C variants by phylogenetic analysis of 5′-untranslated and coding region sequences. J Virol 71:6501–6508.

Nübling CM, Löwer J. 1996. GB-C genomes in a high-risk group, in plasma pools, and in intravenous immunoglobulin. Lancet 347:68.

Radkowski M, Wang LF, Vargas H, Rakela J, Laskus T. 1998. Lack of evidence for GB virus C/hepatitis G virus replication in peripheral blood mononuclear cells. J Hepatol 28:179–183.

Ross RS, Viazov S, Schmitt U, Schmolke S, Tacke M, Ofenloch-Haenle B, Holtmann M, Müller N, Da Villa G, Yoshida CF, Oliveira JM, Szabo A, Paladi N, Kruppenbacher JP, Philipp T, Roggendorf M. 1998. Distinct prevalence of antibodies to the E2 protein of GB virus C/hepatitis G virus in different parts of the world. J Med Virol 54:103–106.

Rubio A, Rey C, Sanchez-Quijano A, Leal M, Pineda JA, Lissen E, Hess G. 1997. Is hepatitis G virus transmitted sexually? J Am Med Assoc 277:532–533.

Saito S, Tanaka K, Kondo M, Morita K, Kitamura T, Kiba T, Numata K, Sekihara H. 1997. Plus- and minus-stranded hepatitis G virus RNA in liver tissue and in peripheral blood mononuclear cells. Biochem Biophy Res Commu 237:288–291.

Sampietro M, Corbetta N, Cerino M, Fabiani P, Ticozzi A, Orlandi A, Lunghi G, Fargion S, Fiorelli G, Cappellini MD. 1997. Prevalence and clinical significance of hepatitis G virus infection in adult beta-thalassaemia major patients. Br J Haematol 97:904–907.

Schreier E, Höhne M, Künkel U, Berg T, Hopf U. 1996. Hepatitis GBV-C sequences in patients infected with HCV contaminated anti-D immunoglobulin and among i.v. drug users in Germany. J Hepatol 25:385–389.

Simons JN, Leary TP, Dawson GJ, Pilot-Matias TJ, Muerhoff AS, Schlauder GG, Desai SM, Mushahwar IK. 1995. Isolation of novel virus-like sequences associated with human hepatitis. Nature Med 1:564–569.

Skidmore SJ, Collingham KE, Harrison P, Neilson JR, Pillay D, Milligan DW. 1997. High prevalence of hepatitis G virus in bone marrow transplant recipients and patients treated for acute leukemia. Blood 89:3853–3856.

Stark K, Bienzle U, Hess G, Engel AM, Hegenscheid B, Schlüter V. 1996. Detection of the hepatitis G virus genome among injecting drug users, homosexual and bisexual men, and blood donors. J Inf Dis 174:1320–1323.

Tanaka E, Alter HJ, Nakatsuji Y, Shih WK, Kim JP, Matsumoto A, Kobayashi M, Kiyosawa K. 1996. Effect of hepatitis G virus infection on chronic hepatitis C. Ann Int Med 125:740–743.

Thomas DL, Nakatsuji Y, Shih JW, Alter HJ, Nelson KE, Astemborski JA, Lyles CM, Vlahov D (1997): Persistence and clinical significance of hepatitis G virus infections in injecting drug users. J Inf Dis 176:586–592.

Toyoda H, Fukuda Y, Hayakawa T, Takamatsu J, Saito H. 1998. Effect of GB virus C/hepatitis G virus coinfection on the course of HIV infection in hemophilia patients in Japan. J AIDS Human Retrovirol 17:209–213.

Tsuda F, Hadiwandowo S, Sawada N, Fukuda M, Tanaka T, Okamoto H, Miyakawa Y, Mayumi M. 1996. Infection with GB virus C (GBV-C) in patients with chronic liver disease or on maintenance hemodialysis in Indonesia. J Med Virol 49:248–252.

Vargas HE, Laskus T, Radkowski M, Poutous A, Wang LF, Lee R, Dodson F, Gayowski T, Singh N, Marino IR, Fung JJ, Zhang-Keck ZY, Kim JP, Rakela J. 1997. Hepatitis G virus coinfection in hepatitis C virus-infected liver transplant recipients. Transplantation 64:786–788.

Wang HL, Jin DY. 1997. Prevalence and genotype of hepatitis G virus in Chinese professional blood donors and hepatitis patients. J Inf Dis 175:1229–1233.

Yashina TL, Favorov MO, Khudyakov YE, Fields HA, Znoiko OO, Shkurko TV, Bonafonte T, Sevall JS, Agopian MS, Peter JB. 1997. Detection of hepatitis G virus (HGV) RNA: Clinical characteristics of acute HGV infection. J Inf Dis 175:1302–1307.

Zuckerman AJ. 1996. Alphabet of hepatitis viruses. Lancet 346:558–559.